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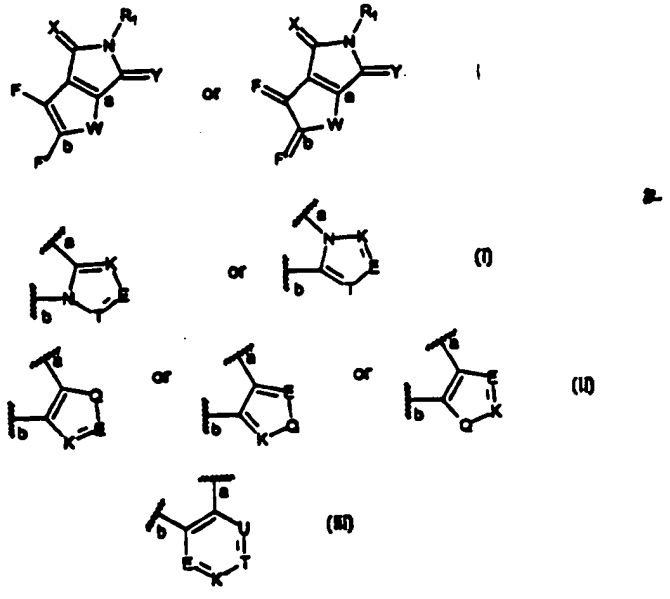
(54) Title: GRANULATIMIDE DERIVATIVES FOR USE IN CANCER TREATMENT



(57) Abstract

Novel granulatimide compounds and pharmaceutical formulations thereof are provided. Compounds of this invention have general formula (I) or (II) wherein are independently R or Z as defined below, or in combination F and F' is Ar1 as defined below; Ar1 is a monocyclic, bicyclic or tricyclic, fully or partially aromatic system containing five or six membered carbocyclic or, oxygen, nitrogen or sulphur containing heterocyclic rings, optionally substituted with R or Z; W is selected from the group consisting f formula (i); (ii) or (iii), wherein the structures are as described in: (i), (ii) and (iii). R and Z are optional substituents as defined in the application.

GRANULATIMIDE DERIVATIVES FOR USE IN CANCER TREATMENT

BACKGROUND

Natural products are a rich source of novel organic compounds, many of which have interesting and desirable biological activities. From extracts of an organism of interest, such as marine invertebrates, methods of chemical separation and analysis may be applied to elucidate the structure of biologically active compounds. These chemical structures then form the basis for synthetic modifications to enhance the desired activity.

Marine ascidians, or sea squirts, have a number of unique secondary metabolites with potent biological activity. Colonial ascidians within the family *Didemnidae* have been particularly prolific, containing nitrogenous amino acid derived metabolites, including various cyclic peptides. The compounds termed didemnimides were isolated from *Didemnum conchyliatum*. These compounds are indole-maleimide-imidazole alkaloids, which could hypothetically be synthesized as a condensation of tryptophan and histidine.

The further study of *Didemna* products, and the characterization of their active agents, is of particular interest for the development of novel therapeutic agents and uses thereof.

Relevant Literature

Didemnimide compounds are described by Vervoot *et al.* (1997) J. Org. Chem. 62:1486-1490. In describing synthesis of such didemnimide compounds, Terpin *et al.* (1998) Tetrahedron 54:1740-1752 reported a ring closing event that may occur upon irradiation or upon recrystallization from methanol of a Boc protected intermediate used in the didemnimide synthesis scheme.

Known G2 checkpoint inhibitors include purine analogues, *e.g.* caffeine, pentoxifylline, 2-aminopurine, 6-dimethylaminopurine; and staurosporine with its derivative UCN-01 (7-hydroxystaurosporine). See, for example, Busse *et al.* (1978) Radiat. Res. 76:292-307; Schlegel (1986) Science 232:1264-1266; Downes *et al.* (1990) J. Cell. Biol. 110:1855-1859; Steinmann *et al.* (1991) P.N.A.S. 88:6843-6847; Andreass n *et al.* (1992) P.N.A.S. 89:2272-2276; Tam *et al.* (1992) Cell Growth Differ. 3:811-817; and Wang *t al.* 1996) J. Nat'l Cancer Inst. 88:956-965.

granulatimide and γ-irradiation do not kill p53+ cells synergistically (method identical to (B) except that MCF-7 cells are used).

Figure 2 is a graph showing G2 checkpoint inhibition by granulatimide.

DETAILED DESCRIPTION OF EMBODIMENTS OF THE INVENTION

Novel granulatimide compounds of Formula I are provided. This invention includes the naturally occurring compounds, granulatimide and iso-granulatimide, in purified or partially purified form, including extracts containing these compounds taken from naturally occurring sources. In one embodiment of the invention, formulations of the compounds in combination with a physiologically acceptable carrier are provided.

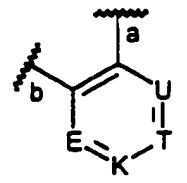
The pharmaceutical formulations are useful as a cytotoxic agent; and to inhibit protein kinases, including those that act to regulate the G2 checkpoint. Such G2 checkpoint inhibition prevents arrest, or releases cells that are arrested at this checkpoint, thereby permitting the cells to proceed to mitosis. This invention also provides the use of compounds of Formula I to sensitize cells to the effects of DNA damaging agents; and the use of such compounds in the formulation of agents, including medicaments, for potentiating the effect of DNA damaging agents on cells.

DEFINITIONS

It is to be understood that this invention is not limited to the particular methodology, protocols, cell lines, animal species or genera, and reagents described, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

As used herein the singular forms "a", "and", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference t "a compound" includes a plurality of such compounds and reference to "the protein" includes reference to one or more prot ins and equivalents thereof known to those skilled in th art, and so forth. All technical and scientific terms

in which K and E are independently selected from the group consisting of: N, CR and CZ, and wherein R, Z and Q are as defined below; and

(iii) 

in which K, E, T and U are independently selected from the group consisting of: N, CR and CZ, and wherein R and Z are as defined below;

R_1, is selected from the group consisting of: R; RCO-; Ar_2CO-; and, Ar_2CH_2-, wherein Ar_2 is an aromatic substituent selected from the group consisting of: phenyl, naphthyl, anthracyl, phenanthryl, furan, pyrrole, thiophene, benzofuran, benzothiophene, quinoline, isoquinoline, imidazole, thiazole, oxazole, and pyridin , and Ar_2 may be optionally substituted with R or Z, wherein R and Z are as defined below;

R is selected from the group consisting of H; and a structural fragment having a saturated or unsaturated linear, branched, or cyclic, skeleton containing one to ten carbon atoms in which the carbon atoms may be optionally substituted with a substituent selected from the group consisting of: -OH; $-OR_3$; $-O_2CR_3$, -SH; $-SR_3$; $-SOCR_3$; $-NH_2$; $-NHR_3$; $-NH(R_3)_2$; $-NHCOR_3$; $NRCOR_3$; -I; -Br; -Cl; -F; -CN; $-CO_2H$; $-CO_2R_3$; -CHO; $-COR_3$; $-CONH_2$; $-CONHR_3$; $-CON(R_3)_2$; -COSH; $-COSR_3$; $-NO_2$; $-SO_3H$; $-SOR_3$; and $-SO_2R_3$, wherein R_3 is a linear, branched or cyclic, one to ten carbon saturated or unsaturated alkyl group;

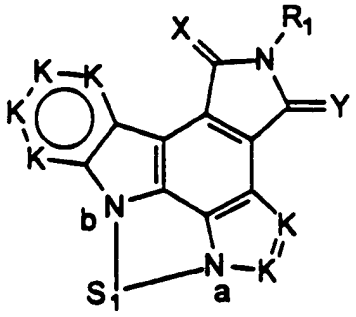
Z is an optional substituent selected from the group consisting of: H; -OH; -OR; $-O_2CR$; -SH; -SR; -SOCR; $-NH_2$; -NHR; $-NH(R)_2$; -NHCOR; NRCOR; -I; -Br; -Cl; -F; -CN- $-CO_2H$; $-CO_2R$; -CHO; -COR; $-CONH_2$; -CONHR; $-CON(R)_2$; -COSH; -COSR; $-NO_2$; $-SO_3H$; -SOR; and, $-SO_2R$;

Q is selected from the group consisting of: NR_1; O; S, and $C(R)_2$; and

X and Y are independently selected from the group consisting of: O; H, OH; and H_2.

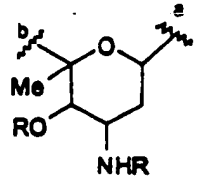
In a preferred embodiment of the invention, F and F' are a bicyclic aromatic system comprising a five-membered and a six-membered ring, having the structure as follows:

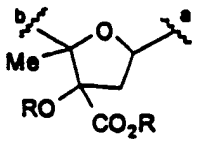
Formula III

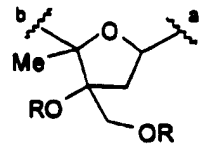


Where S1 is selected from the group consisting of

S_1=

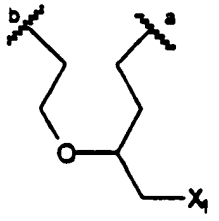




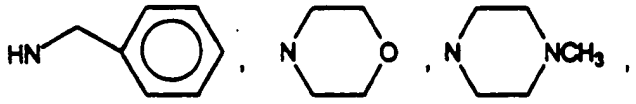


wherein K, X, Y and R_1 are as defined above, and R is an alkyl group of from 1 to 6 carbon atoms, branched or unbranched, linear or circular.

where K, X, Y and R_1 are as defined above, and S_3 is



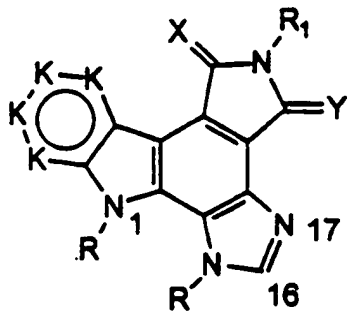
and X_1 is selected from the group of $N(CH_3)_2$, $NHCH_3$, NH_2,  ,



or S_3 is a linear bridge of between five and eight carbon, nitrogen or oxygen atoms, where the bridge carbon atoms may have OR or NR_2 substituents and the bridge nitrogen atoms may have R substituents, where R is as defined above.

Preferred structures for W are the five membered rings described abov , more preferably comprising one or two nitrogen atoms. Preferably, E, K, T and U (where present) are: N or CH. Also, preferably Q is NH. Also preferably, R_1 is: H or CH_3. Also preferably, X and Y are oxygen.

The following are examples of compounds of Formula 1 in which F and F' are Ar_1, with each of K, Q, R and Z being independently selected as defined above.

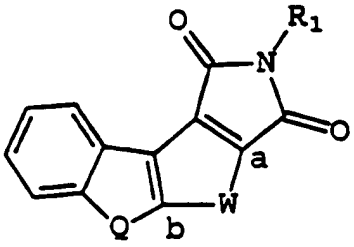


Formula II

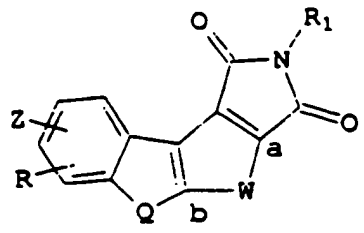
Preferably, excluded as granulatimide compounds of the invention are derivatives comprising a protecting group, *e.g.* Boc, *etc.*, at the position corresponding to 1 in the above scheme.

Preferred compounds of Formula I are as follows, with substituents as defined above or as particularly defined below, where "Me" represents a methyl substituent throughout.

100



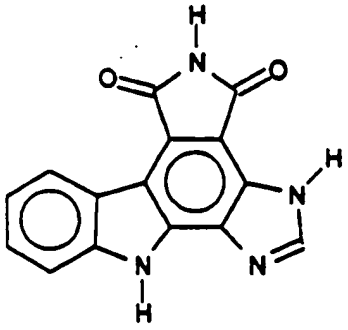
wherein Q=NH; R_1=H; and each of K, E, T = N or CH;



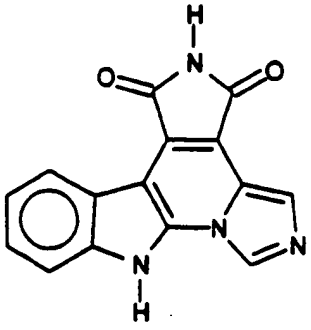
200

wher in Q=NH; R_1=H; and each of K, E, T = N or CH;

In one embodiment of th invention, the granulatimide compound is one of the naturally occurring compounds: granulatimide, or isogranulatimide.



granulatimide



isogranulatimide

Pharmaceutically acceptable salts of the granulatimide compounds also fall within the scope of the compounds as disclosed herein. The term "pharmaceutically acceptable salts" as used herein means an inorganic acid addition salt such as hydrochloride, sulfate, and phosphate, or an organic acid addition salt such as acetate, maleate, fumarate, tartrate, and citrate. Examples of pharmaceutically acceptable metal salts are alkali metal salts such as sodium salt and potassium salt, alkaline earth metal salts such as magnesium salt and calcium salt, aluminum salt, and zinc salt. Examples of pharmaceutically acceptable ammonium salts are ammonium salt and tetramethylammonium salt. Examples of pharmaceutically acceptable organic amine addition salts are salts with morpholine and piperidine. Examples of pharmaceutically acceptable amino acid addition salts are salts with lysine, glycine, and phenylalanine.

Protein Kinases and Inhibitors: These enzymes use the gamma phosphate of ATP or GTP to generate phosphate monoesters utilizing protein alcohol groups on serine or threonine, and/or protein phenolic groups (tyrosine) as phosphate group acceptors. They are related by virtue of their homologous kinase domains, which consist of 200-300 amino acid residues. The kinase domains impart th catalytic activity by binding and orientation of the phosphate donor as a complex with divalent cation; binding and orientation of the polypeptide substrate; and transfer of the γ-phosphate from the ATP or GTP to the acceptor hydroxyl residu .

G2 checkpoint: Normal cells respond to DNA damage in one of two ways, depending upon their type and the degree of damage. The cells may activate an apoptotic pathway leading to suicide of the cell and its removal or, survival of a damaged cell may be promoted by activating checkpoints that temporarily halt the normal cycle of growth and division to allow time for DNA repair. The checkpoints operate during the G1 phase of the cycle so that DNA is repaired before it is replicated in the S phase; and, during the G2 phase so that DNA is repaired before chromosomes are segregated in the mitosis phase (M).

The subject granulatimide compounds are active in the inhibition of the G2 checkpoint. They are of interest as therapeutic reagents to treat cancer, *etc.* Administration of the subject compounds will release cells from the G2 checkpoint; and can result in apoptosis of the treated cells. Preferably the treated cells are unable to activate the G1 checkpoint. Many human tumors have mutations in the protein p53. As a result, they are unable to activate the G1 checkpoint, but can still utilize the G2 checkpoint. Inhibiting the checkpoint would drive tumor cells into mitosis, thereby increasing the effectiveness of cytotoxic agents that act by damaging DNA, *e.g.* cisplatin, bleomycin, and etoposide; and radiotherapy.

Over 50% of human cancers exhibit a loss of function of the protein p53. Cells with mutated p53 are unable to activate the G1 checkpoint in response to DNA damage. However, the G2 checkpoint (although usually weaker than in normal cells) still provides an opportunity to repair the DNA damage before cell division. Inhibition of the G2 checkpoint alone generally does not have a strong effect on normal cells, or tumor cells that retain normal G1 checkpoint activity. G2 checkpoint inhibitors used in combination with a DNA damaging agent will significantly increase the killing of cells that cannot activate the G1 checkpoint.

Throughout this specification, the term "G2" or G2 phase" means the phase of the cell cycle between the end of DNA synthesis and the beginning of mitosis. A cell in G2 has an interphase nucleus as determined by microscopy, and duplicated DNA (usually determined by flow cytometry).

G2 checkpoint Inhibitor: Throughout this specification, the term "G2 checkpoint inhibitor" means a substance which is capable of releasing a cell from

animals. Specific examples of suitabl cells are th following: cells which have incorporated the human papillomavirus type-16 E6 gene, cell lines which are mutant for p53 function including Burkitt's human lymphoma cell lin CA46 and the human colon carcinoma cell lin HT-29 (CA46 and HT-29 are available from the American Type Culture Collection). Cell lines with a genetic deficiency which disrupts the G1 checkpoint other than by disruption of p53 may also be employed in this assay.

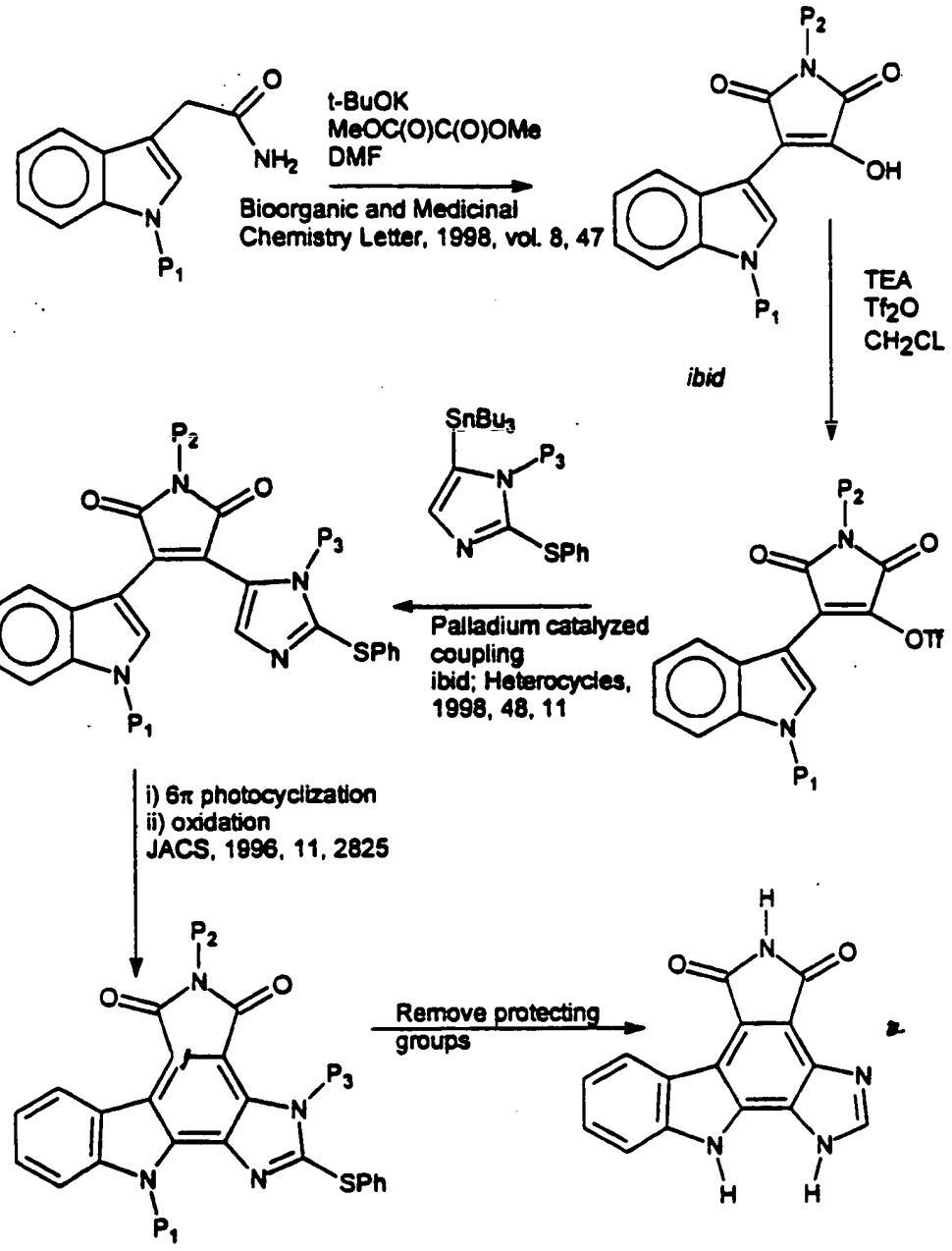
Once a cell line incapable of G1 arrest is chosen, conditions for arresting a majority of the cells at G2 in response to a DNA damaging agent are optimized by determining appropriate culture conditions, incubation time, and type and dosage of DNA damaging agent. Preferably, at least 50% of the cells in a culture will be arrested at G2 in response to the DNA damaging agent. Maximizing the proportion of cells in the population which are arrested at G2 will reduce the background signal.

Once the conditions for G2 arrest in the cell culture are established, the assay may be carried out in one of two ways. First, the cells may be arrested at G2 in response to the DNA damaging agent and then treated with the sample to determine whether there is release from G2 arrest or, the cells may be treated with the sample prior to the time when the majority of the cells would be expected to b arrested at G2 and determine whether the cells are prevented from G2 arrest.

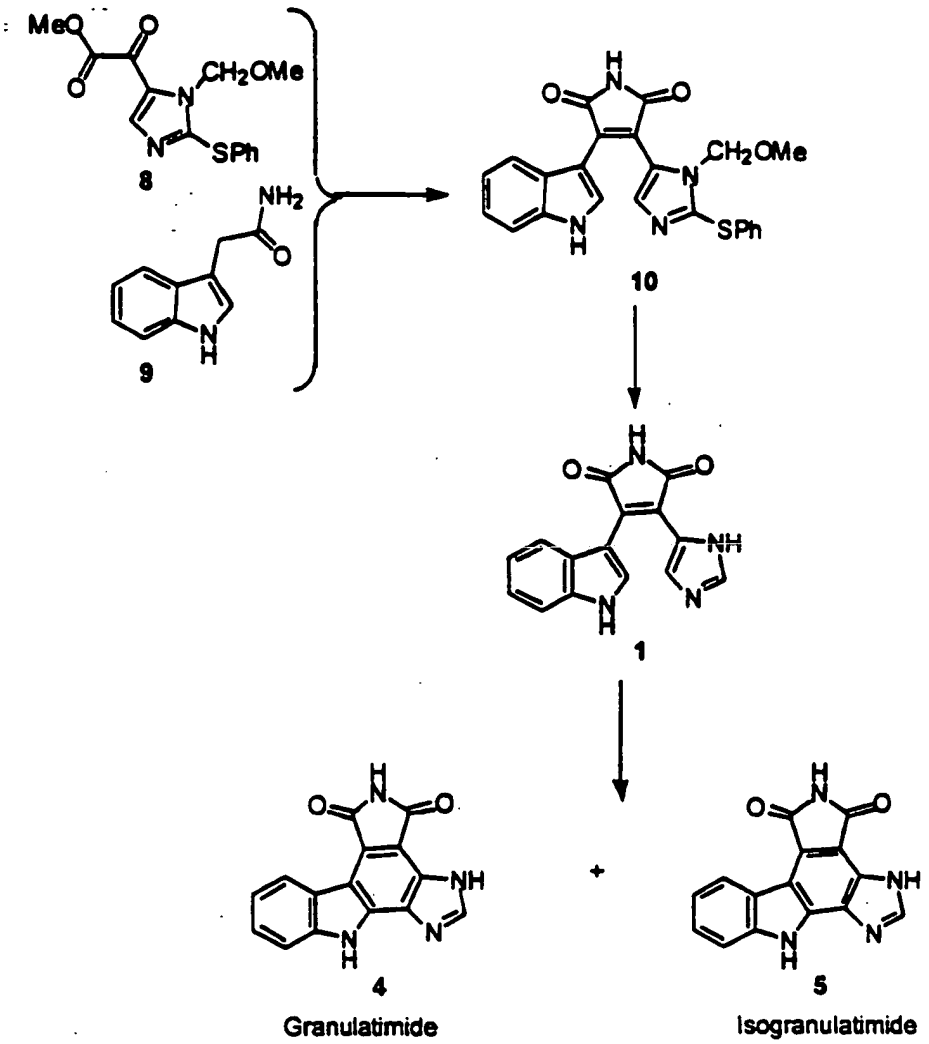
Release from G2 arrest or prevention of G2 arrest is detected by a quantitative determination of the cells which proceed to mitosis. The assay culture is treated with an agent which will arrest such cells in mitosis. Such agents include microtubule depolymerizing agents that arrest cells in metaphase, such as nocodazole. This will prevent cells from exiting mitosis and entering the next cell cycle.

In the assay, determination of the cells which proceed to mitosis is carried out using any of the known immunological methods by employing antibodies which have specificity for mitotic cells. Monoclonal antibodies demonstrating such specificity are known and include MPM-2 which was raised against mitotic HeLa cells and recognizes phospho-epitopes that are highly conserved in mitotic proteins of all eukaryotic species. Other examples are the mon clonal antibodies recognizing phospho-epitopes in the paired helical filament prot ins (PHF) found in

An exemplary synthetic process is as follows, where P_1, P_2 and P_3 are suitable protecting groups. Th starting indole compound (appropriately substituted by R and or Z) may be made by methods known in the art.



An alternative synthesis scheme, outlined below, involves essentially a single coupling step to produce a didemnimide compound followed by cyclization to produce a compound of Formula I, wherein F, F', X and Y are as defined for Formula I, and W is as defined for Formula I joined to the reactant at (a) and not (b):



Alternatively, compounds of Formula I can be produced from granulatimide or isogranulatimide which may be synthesized as described above or obtained from natural sources as described in the Examples. The following schematic describes modification of granulatimide to produce derivatives having different X and Y substituents. The ratio of II to III will be dictated by the choice of protecting groups P_1, and P_2 or P_3, which may be (for example) Cbz, R or H. A protecting group will be used at either P_2 or P_3, depending upon the position of the double bond in the ring. The chemistry is described in Link, *et al.* (1996) J. American Chemical Society 118:2825, at p. 2832.

All Z and R groups can be added to aromatic carbons in W and Ar_1 prior to the coupling step to prepare the maleimide intermediates. All R, RCO, Ar_2CO, and

PHARMACEUTICAL FORMULATIONS

The compounds of this inv ntion can be incorporated into a variety of formulations for therapeutic administration. More particularly, the compounds of the present invention can be formulated into pharmaceutical compositions by combination with appropriate pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. As such, administration of the compounds can be achieved in various ways, including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, transdermal, intracheal, *etc.*, administration. The active agent may be systemic after administration or may be localized by the use of regional administration, intramural administration, or use of an implant that acts to retain the active dose at the site of implantation.

In pharmaceutical dosage forms, the compounds may be administered in the form of their pharmaceutically acceptable salts. They may also be used in appropriate association with other pharmaceutically active compounds. The following methods and excipients are merely exemplary and are in no way limiting.

For oral preparations, the compounds can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

The compounds can be formulated into preparations for injections by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

The compounds can be utilized in aerosol formulation to be administered via inhalation. The compounds of the present invention can b formulated into

tonicity adjusting agents, stabilizers, wetting agents and the like, are readily availabl to th public.

The combined use of granulatimide compounds and other cytotoxic agents has the advantages that the required dosages for the individual drugs is lower, and the effect of the different drugs complementary. Depending on the patient and condition being treated and on the administration route, the granulatimide compounds may be administered in dosages of 0.1 μg to 10 mg/kg body weight per day. The range is broad, since in general the efficacy of a therapeutic effect for different mammals varies widely with doses typically being 20, 30 or even 40 times smaller (per unit body weight) in man than in the rat. Similarly the mode of administration can have a large effect on dosage. Thus for example oral dosages in the rat may be ten times the injection dose. Higher doses may be used for localized routes of delivery.

A typical dosage may be a solution suitable for intravenous administration; a tablet taken from two to six times daily, or one time-release capsule or tablet taken once a day and containing a proportionally higher content of active ingredient, *etc.* The time-release effect may be obtained by capsule materials that dissolve at different pH values, by capsules that release slowly by osmotic pressure, or by any other known means of controlled release.

Those of skill will readily appreciate that dose levels can vary as a function of the specific compound, the severity of the symptoms and the susceptibility of the subject to side effects. Some of the specific compounds are more potent than others. Preferred dosages for a given compound are readily determinable by those of skill in the art by a variety of means. A preferred means is to measure the physiological potency of a given compound.

For use in the subject methods, the granulatimide compounds may be formulated with other pharmaceutically active agents, particularly other anti-metastatic, anti-tumor or anti-angiogenic agents. Angiostatic compounds of interest include angiostatin, endostatin, carboxy terminal peptides of collagen alpha (XV), *etc.* Cytotoxic and cytostatic agents of interest include adriamycin, alkeran, Ara-C, BICNU, busulfan, CNNU, cisplatinum, cytoxan, daunorubicin, DTIC, 5-FU, hydrea, ifosfamide, methotrexate, mithramycin, mitomycin, mitoxantron , nitrogen mustard, velban, vincristine, vinblastine, VP-16,

therapy, th active agents can be delivered tog ther or separately, and simultaneously or at different times within the day.

The susceptibility of a particular tumor cell to killing with the combined therapy may be determined by *in vitro* testing. Typically a culture of the tumor cell is combined with a combination of a cytotoxic compound and a granulatimid compound at varying concentrations for a period of time sufficient to allow th active agents to induce cell death, usually between about one hour and one week. For *in vitro* testing, cultured cells from a biopsy sample of the tumor may be used. The viable cells left after treatment are then counted.

The dose will vary depending on the specific cytotoxic agent utilized, type of tumor, patient status, *etc.*, at a dose sufficient to substantially ablate the tumor cell population, while maintaining patient viability. In some cases therapy may be combined with stem cell replacement therapy to reconstitute the patient hematopoietic function. Treatment will generally be continued until there is a substantial reduction, *e.g.* at least about 50%, decrease in the tumor burden, and may be continued until there are essentially no tumor cells detected in the body.

All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing, for example, the compounds and methodologies that are described in the publications which might be used in connection with the presently described invention. The publications discussed above and throughout the text are provided solely for their disclosure prior to th filing date of the present application. Nothing herein is to be construed as an admission that the inventors are not entitled to antedate such disclosure by virtu of prior invention.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the subject invention, and are not intended to limit the scope of what is regarded as the invention. Efforts have been made to ensure accuracy with respect to th numbers used (*e.g.* amounts, temperature, concentrations, *etc.*) but som experimental errors and deviations should be allowed for. Unless otherwis indicated, parts are parts by weight, molecular w ight is av rage molecular w ight, temp rature is in degrees centigrad ; and pressure is at or near atmospheric.

peroxide was added for 1 hour at room temperature and th plat s were read at 405 nm using a BioTek™ plate reader. Positive controls treated with 2 mM caffeine gave absorbance readings of about 1.0.

G2 checkpoint inhibition was detected in extracts from the ascidian *Didemnum granulatum* (Subphylum Urochordata, Class Ascidiacea) collected from rocky, shallow water marine habitats along the coastline of southern Brazil.

Collection and Extraction of D. Granulatum.

Didemnum granulatum (85 g wet wt.) was collected during the summer of 1995 at depths of 1 m at the rocky shore of Araca beach, Sao Sebastiao (Sao Paulo state, southeastern Brazil). A second collection was made at the Arquipelago do Arvoredo (Santa Catarina state, southern Brazil, 150 g wet wt.) and in the Sao Sebastiao Channel (Sao Paulo state, 185 g wet wt.) during November 1997. Freshly collected animals were stored in ethanol at –20°C. Animals obtained from the different collections were processed separately as follows: after decantation of ethanol, the animal was blended and extracted three times with methanol. The ethanol and methanol extracts were combined, filtered and evaporated *in vacuo* to give a gummy residue. The bulk of the residue was dissolved in 8:2 MeOH-CH_2Cl_2 and filtered for elimination of inorganic salts while small amounts of the residue were dissolved in DMSO for use in the G2 checkpoint inhibition assay described above.

Isolation of Active Compounds.

Methanol extracts from the Brazilian ascidian *D. granulatum* were fractionated by gel filtration on Sephadex LH-20 (eluent: MeOH) followed by reversed phase HPLC (eluent: acetonitrile/0.05% trifluoroacetic acid (1:1)) affording a series of eight fractions having different constituents by TLC. Only one fraction exhibited G2 checkpoint inhibition activity, which provided the novel alkaloid compounds later termed iso-granulatimide and granulatimide. Other fractions contained various forms of the alkaloid didemnimide which were inactive. Didemnimide A and D were identified by comparison of NMR and MS data with literature values (Vervoort, H.C. *et al. [supra]*).

indole NH. Th absence of a resonance in the ^{1}H NMR spectrum of the compound that may be assigned to the indole H-2 or H-3 protons indicated the presence of substituents at C-2 and C-3.

Very broad resonances at δ 8.10 and δ 9.12 in the ^{1}H NMR spectrum were assigned to the imidazole moiety. The broadness of the imidazole resonances was attributed to tautomeric equilibrium of the NH protons. The large chemical shift observed for the H-4 resonance (δ 8.51) in the new compound relative to the chemical shift observed for the H-4 resonance in didemnimide A (δ 7.07) was attributed to a neighbouring group effect from a C-9 maleimide carbonyl.

As described above, the new compound was predicted to be a polycyclic aromatic differing from didemnimide A by the presence of a bond between the C-2 carbon of the indole fragment and either C-14 or N-17 of the imidazole fragment. In the former case, the compound would be granulatimide as described herein and in the latter case, isogranulatimide. The broadness of the imidazole resonances in the NMR spectra initially made use of NMR ineffective in distinguishing the two compounds. Therefore, both granulatimide and isogranulatimide were synthesized as described herein and compared to the compound derived from the natural source which revealed the compound to be isogranulatimide. TLC analysis (CH_2Cl_2 - MeOH 9:1; UV at 254nm) of pooled active fractions from the natural extract using synthetic granulatimide as a reference revealed presence of the latter compound in an amount substantially less than isogranulatimide. However, it was determined that the synthetic granulatimide is very insoluble in most common organic solvents including ethanol and methanol and was therefore not efficiently extracted fr ,m the natural source in the procedure described above.

Granulatimide is soluble in DMF or DMSO and one of the latter solvents is recommended for extraction of granulatimide from the natural source.

Table 1 provides comparative ^{1}H-NMR data determined using synthetic compounds as recorded in DMSO-d_6.

would preferentialliy kill p53- tumors in humans treated with DNA damaging therapy.

As is shown in Figure 2, granulatimide may be more potent as a G2 checkpoint inhibitor (IC_{50} = 1.3μm) as compared to isogranulatimide.

Example 2

Synthesis of Isogranulatimide and Granulatimide.

Throughout these examples, TLC was carried out by using commercial aluminum-backed silica gel 60 plates. Flash chromatography was carried out with 230-400 mesh silica gel (E. Merck). THF was distilled from sodium/benzophenone and CH_2Cl_2 was distilled from CaH_2. Commercial EtOH (reagent grade) and MeCN (HPLC grade) were used without further purification. Molecular sieves were dried under vacuum with heating for 5 hours prior to use. All reactions were carried out under an atmosphere of dry argon using glassware that had been thoroughly flame dried.

Methyl 2-(1-methoxymethyl-2-phenylthioimidazol-5-yl)glyoxylate (8).

To a cold (-78°C), stirred solution of 1-methoxymethyl-2-phenylthioimidazole (Ohta, S. *et al.* (1992) Chem. Pharm. Bull. 40:2681) (340 mg, 1.55 mmol) in dry THF (8.0 mL) was added a solution of n-BuLi (1.47 M in hexanes, 1.26 mL, 1.85 mmol) and the mixture was stirred for 1 hour. A solution of dimethyl oxalate (540 mg. 4.58 mmol) in dry THF (2.0 mL) was added and the mixture was stirred at -78°C for an additional 1.25 hours. The reaction mixture was treated with saturated aqueous NH_4Cl (5.0 mL) and Et_2O (20 mL). The phases were separated and th aqueous layer was extracted with Et_2O (25 mL). The combined organic extracts were washed (brine, 10 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (35 g of silica gel, 1:1 petroleum ether-Et_2O) of the crude material afforded 305 mg (66%) of 8 as a beige solid that exhibited mp 59-60 °C; IR (KBr) 1729, 1657, 1305, 1273, 1167, 1114, 784 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 8.23 (s, 1 H), 7.52-7.54 (m. 2 H), 7.37-7.38 (m, 3 H), 5,81 (s, 2 H), 3.91 (s, 3 H), 3.36 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.2, 161.6, 154.1, 145.4, 145.3, 133.2, 129.5, 129.2, 128.7, 75.8, 56.5, 53.0; HREIMS calcd for $C_{14}H_{14}N_2O_4S$ 306.0674, found 306.0674.

J = 8.0 Hz), 5.02 (s, 2 H), 3.07 (s, 3 H); ^{13}C NMR (100 MHz) δ 171.8, 171.7, 140.5, 136.5, 134.3, 132.9, 131.8, 124.3, 122.4, 121.7, 120.5, 120.3, 118.4, 112.3, 104.7, 76.4, 55.6; HREIMS calcd for C17H14N4O3 322.1066, found 322.1066.

Synthesis of Didemnimide A (1).

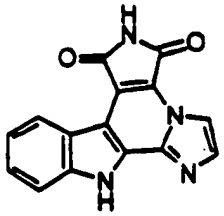
To a stirred suspension of 11 (67.4 mg, 0.209 mmol) in CH_2Cl_2 (15.0 mL) at room temperature was added a solution of BBr_3 in CH_2Cl_2 (1.0 M, 2.1 mL, 2.1 mmol) and the deep blue solution was stirred for 5 hours. The mixture was treated with saturated aqueous $NaHCO_3$ (10 mL) and EtOAc (20 mL) and then was stirred for 0.25 hours. The phases were separated and the aqueous layer was extracted with EtOAc (2 x 15 mL). The combined organic extracts were washed (brine, 10 mL), dried ($MgSO_4$) and concentrated. Flash chromatography (10 g of silica gel, 15:1 CH_2Cl_2-MeOH) of the crude material afforded 15.8 mg of recovered 11 as well as 45.0 mg (77%, 100% based on recovered starting material) of 1 as an orange solid ^1H NMR (400 MHz, major tautomer) δ 12.45 (br s, 1 H), 11.66 (br s, 1H), 10,87 (br s, 1 H), 8.05 (s, 1 H), 7.71 (br s, 1H), 7.68 (br s, 1 H), 7.39 (br d, 1 H, J = 7.8 Hz), 7.07 (br m, 2 H), 6.87 (br m, 1 H); ^{13}C NMR (100 MHz) δ 172.8, 172.6, 136.1, 135.9, 130.9, 130.5, 126.0, 126.0, 121.7, 121.3, 119.7, 119.2, 112.2, 111.5, 105.0; HREIMS calcd for $C_{15}H_{10}N_4O_2$ 278.0804, found 278.0804.

Granulatimide (4) and Isogranulatimide (5).

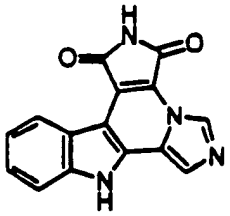
To a solution of 1 (12.9 mg, 0.046 mmol) in MeCN (5.0 mL) was added a catalytic amount of palladium-on-carbon (10% Pd) and the resulting mixture was sparged with argon for 0.5 hours. The mixture was irradiated (450 Watt Hanovia medium pressure mercury vapor lamp, quartz reaction vessel) for 6.5 hours. Th reaction mixture was filtered through Celite, the Celite was washed with DMF (10 mL), and the combined filtrate was concentrated. The remaining material was taken up in EtOAc (30 mL) and the resultant solution was washed (brine, 4 x 20 mL), dried ($MgSO_4$) and concentrated. Flash chromatography (20 g of silica gel, 10:1 CH_2Cl_2-MeOH) of the crude material afforded 11.7 mg (91%) of 4 as a yellow solid and 1.0 mg (8%) of 5 as a red solid. Final purification was achieved on RPHPLC (1:1 CH_3CN-0.05% TFA). Granulatimide (4): ^1H NMR (400 MHz) see Table 1; UV (MeOH) 230, 270, 285, 295, and 380 nm; HREIMS calcd for

Exampl 4

Synthesis of Granulatimide Compounds.

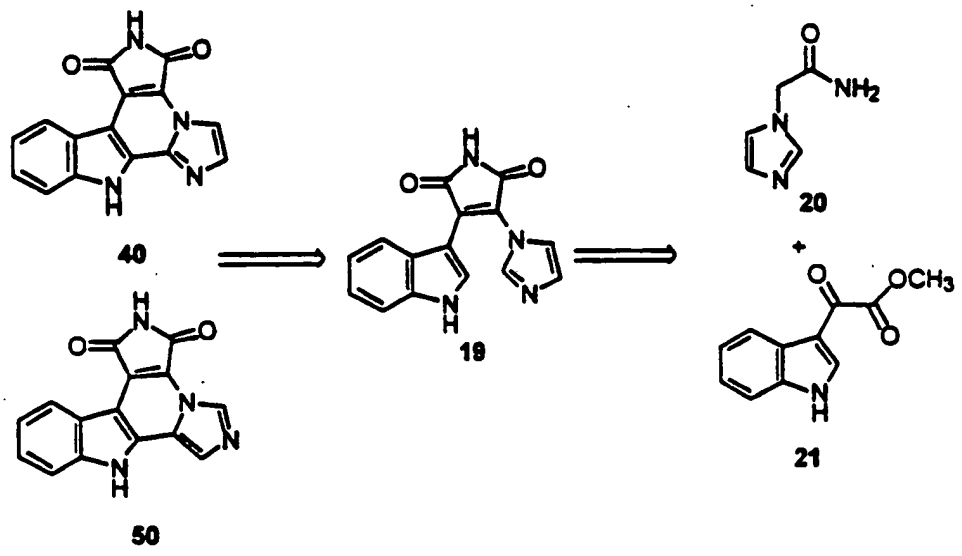


40



50

Retrosynthesis



solid that exhibited: ^{1}H NMR (400 MHz) δ 12.40 (bs, 1 H), 8.44 (d, 1 H, J = 3.0 Hz), 8.15 (d, 1 H, J = 7.0 Hz), 7.54 (d, 1 H, J = 7.6 Hz), 7.24-7.31 (m, 2 H), 3.88 (s, 3 H); ^{13}C NMR (100 MHz) δ 178.7, 163.9, 138.3, 136.7, 125.4, 123.8, 122.8, 121.1, 112.7, 112.4, 52.5; HREIMS calcd for $C_{11}H_9NO_3$ 203.0582, found 203.0582.

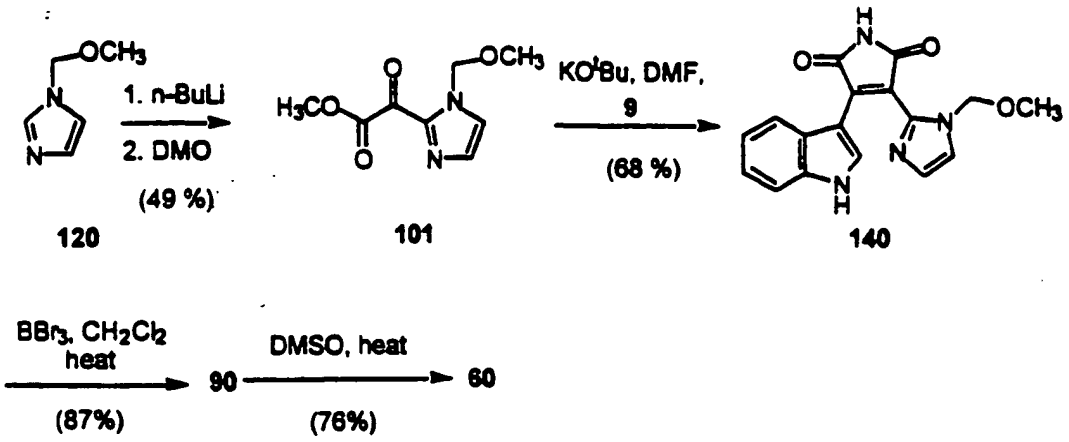
3-[4-(1H-imidazol-1-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indole (19).

To a stirred solution of *t*-BuOK (140 mg, 1.25 mmol) in DMF (2.0 mL) at 0 °C was added a solution of **20** (31.1 mg, 0.249 mmol) and methyl indole-3-glyoxylate (**21**) (101.1 mg, 0.498 mmol) in DMF (3.0 mL). The reaction mixture was stirred for 12 h at rt and then heated to 45 °C for 3 h. The reaction mixture was then cooled and treated with saturated aqueous NH_4Cl (5 mL) and EtOAc (20 mL). The phases were separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic extracts were washed with brine (4 x 10 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (25 g of silica gel, 10:1 then 6:1 CH_2Cl_2-MeOH) of the crude material afforded 54.5 mg (79%) of **19** as a red solid that exhibited IR (KBr) 3121, 2729, 1767, 1719, 1651, 1495, 1340, 1239, 746 cm^{-1}; ^{1}H NMR (400 MHz) δ 10.75 (bs, 1 H), 10.00 (bs, 1 H), 8.03 (d, 1 H, J = 3.1 Hz), 7.78 (s, 1 H), 7.48 (d, 1 H, J = 8.2 Hz), 7.29 (s, 1 H), 7.11 (dd, 1 H, J = 8,8 Hz), 7.04 (s, 1 H), 6.80 (dd, 1 H, J = 8,8 Hz), 6.21 (d, 1 H, J = 7.6 Hz); ^{13}C NMR (100 MHz) δ 170.4, 168.4, 137.8, 136.4, 131.6, 128.8, 125.8, 124.3, 124.1, 122.5, 120.6, 120.1, 119.6, 112.3, 102.3; HREIMS calcd for $C_{15}H_{10}N_4O_2$ 278.0804, found 278.0804.

RAB-009 (40) and RAB-010 (50).

A solution of **19** in t-BuOH and MeCN was sparged with argon for 0.5 h. The yellow mixture was then irradiated (450 Watt Hanovia medium pressur mercury vapour lamp, quartz reaction vessel) for 4 h. The reaction mixture was concentrated and redissolved in acetone (10 mL). To the crude reaction product in acetone at rt was added 10 X $CuCl_2$ and the reaction mixture was stirred, exposed to air, overnight. The solvent was then removed in vacuo and the residue was taken up in DMF (10 mL) and filtered through celite. The filtrate was then diluted

Synth tic Scheme.



Methyl 2-(1-methoxymethyl-1H-imidazol-2-yl)-glyoxylate (101).

To a cold (-48 °C), stirred solution of 1-methoxymethyl-1*H*-imidazole (**120**) (335 mg, 2.99 mmol) in dry THF (15.0 mL) was added a solution of *n*-BuLi (1.50 M in hexanes, 2.30 mL, 3.45 mmol) and the mixture was stirred for 1 h. The reaction mixture was then cooled to -78°C and added to a solution of dimethyloxalate (1.17 g, 9.9 mmol) in THF (10.0 mL) at -78°C. The mixture was stirred at -78°C for an additional 0.33 h. The reaction mixture was treated with saturated aqueous NH_4Cl (5 mL) and EtOAc (15 mL). The phases were separated and the aqueous layer was extracted with EtOAc (2 x 20 mL). The combined organic extracts were washed with brine (10 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (50 g of silica gel, 50:1 CH_2Cl_2-MeOH) of the crude material afforded 290 mg (49%) of **101** as an oil that exhibited IR (KBr) 2957, 1744, 1679, 1412, 1276, 1215, 1109, 1005 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.38 (s, 1 H), 7.33 (s, 1 H), 5.71 (s, 2 H), 3.96 (s, 3 H), 3.33 (s, 3 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 177.9, 164.3, 140.6, 132.9, 126.9, 78.8, 57.5, 53.4; HREIMS calcd for $C_8H_{10}N_2O_4$ 198.0641, found 198.0641.

*3-[4-(1-m thoxymethyl-1H-imidazol-2-yl)-2,5-dioxo-2,5-dihydro-1H-pyrrol-3-yl]-indole (140).*To a stirred solution of **101** (142.0 mg, 0.717 mmol) and indol

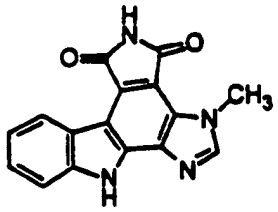
RAB-007 (60).

A stirred solution f **90** (14.4 mg, 0.052 mmol) in DMSO (5 mL) was heated to 120 °C for 8 h. Th reaction mixture was cooled to rt, diluted with EtOAc (30 mL), washed with brine (3 X 10 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (20 g of silica gel, 20:1 then 10:1 CH_2Cl_2-MeOH) of the crude material afforded 11.0 mg (76%) of **60** as an orange solid that exhibited IR (KBr) cm^{-1} 2600-3400, 1755, 1717, 1631, 1591, 1575, 1464, 1376, 1336, 1232, 751; ^1H NMR (400 MHz) δ 13.36 (bs, 1 H), 11.13 (bs, 1 H), 8.63 (d, 1 H, *J* = 7.7 Hz), 8.40 (s, 1 H), 7.93 (s, 1 H), 7.69 (d, 1 H, *J* = 8.2 Hz), 7.48 (dd, 1 H, *J* = 7,7 Hz), 7.38 (dd, 1 H, *J* = 7,7 Hz); HREIMS calcd for $C_{15}H_8N_4O_2$ 276.0647, found 276.0647.

In the G2 checkpoint inhibition assay, compound **60** had an IC_{50} of 2 μM.

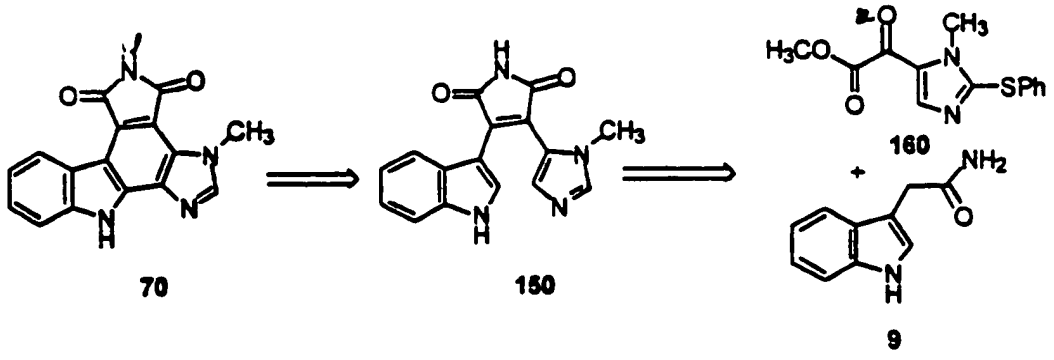
Example 6

Synthesis of Cyclodidemnimide C (70).



70

Retrosynthesis.



70 **150** **160** **9**

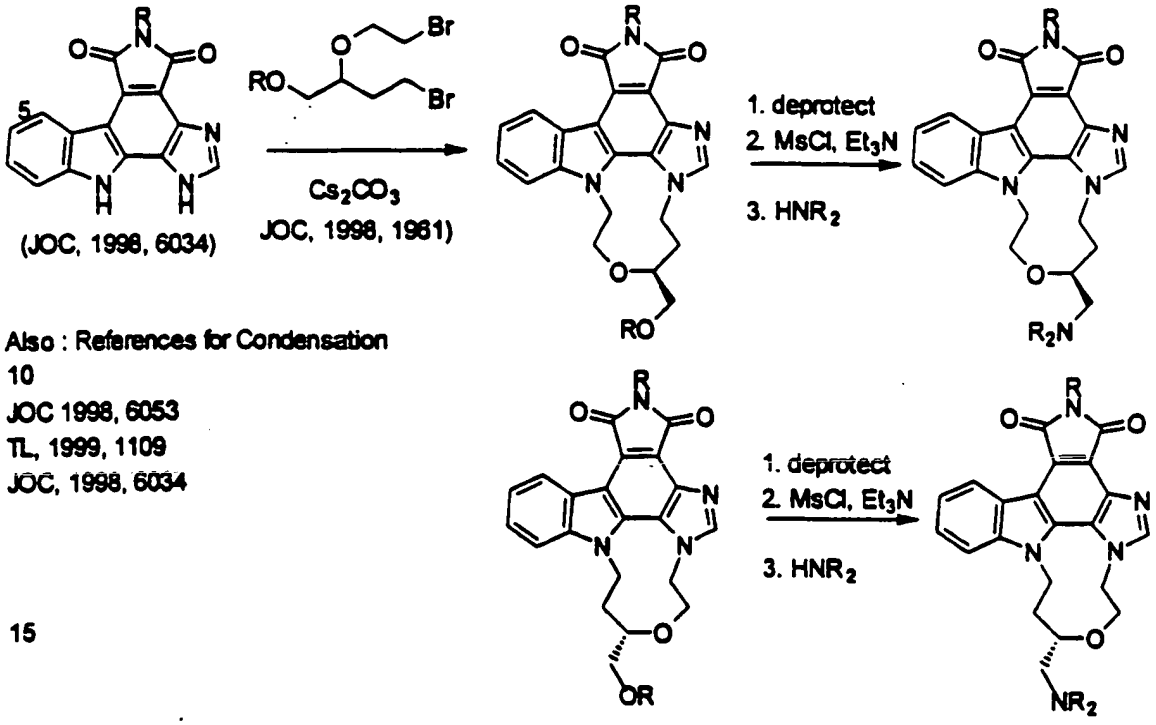
at rt was added sequentially 4A molecular seives (~ 50 mg) and a solution of **160** (28 mg, 0.10 mmol) and indole-3-acetamide **9** (22 mg, 0.13 mmol) in DMF (1.5 mL). Th reaction mixture was heated to 40 C and stirred for 48 h. The dark purple solution was treated with hydrochloric acid (1 N, 1.0 mL) and EtOAc (20 mL). The phases were separated and the aqueous layer was extracted with EtOAc (2 X 20 mL). The combined organic extracts were washed with brine (4 x10 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (20 g of silica gel, 30:1 CH_2Cl_2-MeOH) of the crude material afforded 35 mg (88%) of **170** as a red solid that exhibited; IR (KBr) 2500-3600, 1760, 1713, 1631, 1581, 1441, 1339, 744 cm^{-1}; ^1H NMR (400 MHz) δ 12.06 (bs, 1 H), 11.19 (bs, 1 H), 8.09 (d, 1 H, *J* = 2.1 Hz), 7.46 (d, 1 H, *J* = 8.0 Hz), 7.14-7.31 (m, 6 H), 7.03 (d, 1 H, *J* = 7.6 Hz), 6.81 (dd, 1 H, *J* = 8,8 Hz), 6.47 (d, 1 H, *J* = 8.1 Hz), 3.07 (s, 1 H); ^{13}C NMR (100 MHz) δ 171.6, 171.4, 138.6, 136.4, 134.6, 133.7, 132.7, 131.8, 129.4, 127.4, 126.7, 126.6, 124.5, 120.8, 119.5, 117.9, 112.4, 104.8, 32.4; HREIMS calcd for $C_{22}H_{16}N_4O_2S$ 400.0994, found 400.0994.

*Didemnimide C (**150**).*

To a refluxing solution of **170** (46.7 mg, 0.117 mmol) in EtOH (10 mL) was added Raney Ni (W-2, 50% slurry in water, ~120 mg) and the suspension was refluxed for 1 h. The reaction mixture was then cooled to rt and filtered through Celite. The Celite was washed with DMF (6 mL) and MeOH-TFA (100:1, 30 mL) and the combined organic washes were concentrated. The organic concentrate was then diluted with EtOAc (40 mL) and washed with $NaHCO_3$ (4 x 10 mL) brine (3 x 20 mL), dried ($MgSO_4$) and concentrated. Flash chromatography (25 g of silica gel, 10:1 CH_2Cl_2-MeOH) of the crude material afforded 26.4 mg (77%) of **150** as an orange solid that exhibited; IR (KBr) 3163, 3041, 1766, 1702, 1538, 1345, 1236, 1221, 748 cm^{-1}; ^1H NMR (400 MHz) δ 11.97 (bs, 1 H), 11.09 (bs, 1 H), 8.06 (d, 1 H, J = 2.6 Hz), 7.69 (bs, 1 H), 7.44 (d, 1 H, J = 8.1 Hz), 7.10 (dd, 1 H, J = 8,8 Hz), 6.78 (dd, 1 H, J = 8,8 Hz), 6.40 (d, 1 H, J = 8.1 Hz), 3.17 (s, 1 H); ^{13}C NMR (100 MHz) δ 171.9, 171.7, 140.3, 136.4, 133.9, 132.0, 131.5, 124.7, 122.3, 120.5, 119.7, 112.2, 105.0, 32.0; HREIMS calcd for $C_{16}H_{12}N_4O_2$ 292.0960, found 292.0960

Exampl 8

Synth sis of Compounds related to granulatimid .



(JOC, 1998, 6034)

JOC, 1998, 1961)

Also : References for Condensation

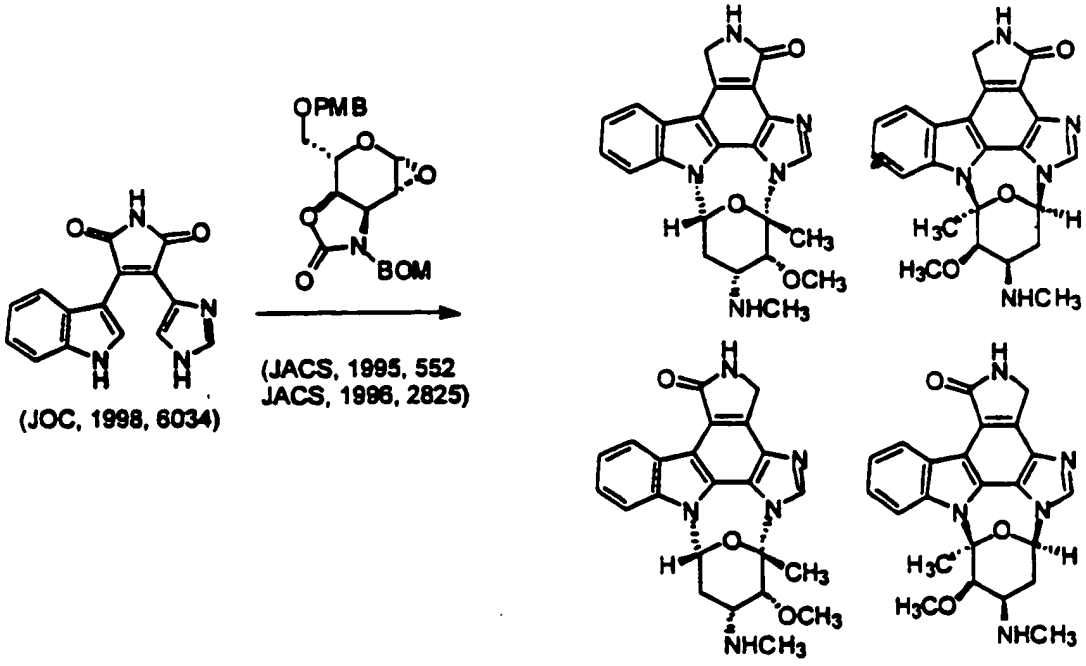
JOC 1998, 6053

TL, 1999, 1109

JOC, 1998, 6034

Example 9

Synthesis of Compounds related to Staurosporine.



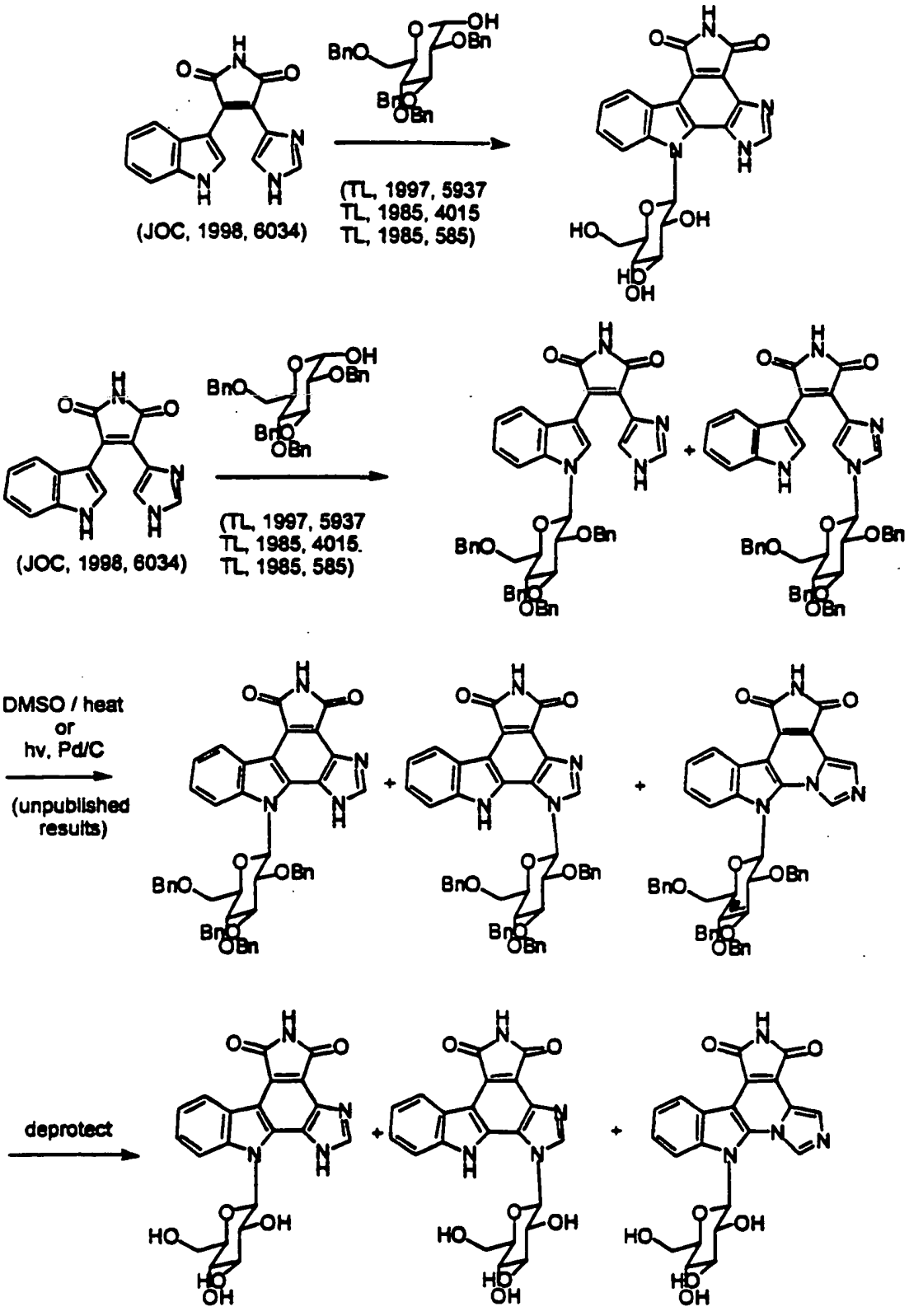
(JOC, 1998, 6034)

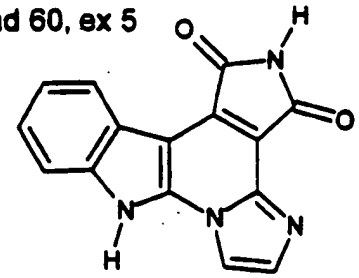
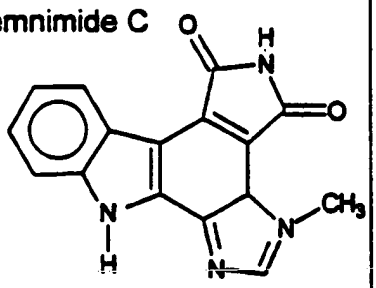
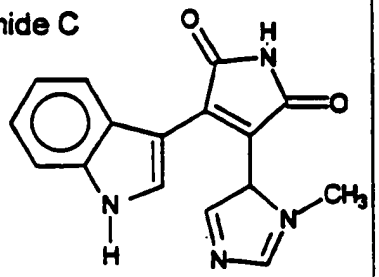
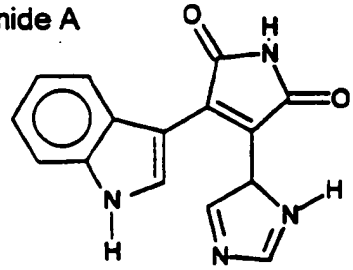
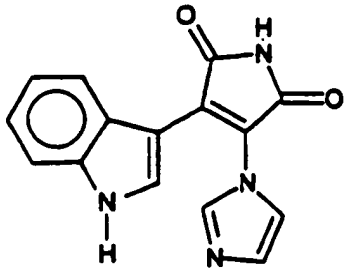
(JACS, 1995, 552

JACS, 1996, 2825)

Example 10

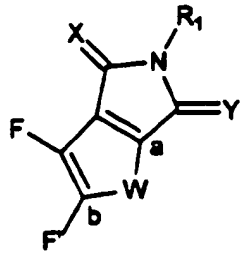
Synthesis of Compounds related to Rebeccamycin.



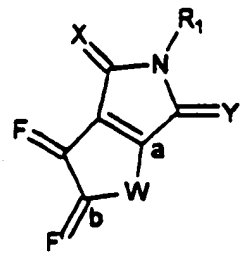
Compound 60, ex 5 RAB-007 	6 μM	17 μM	5 μM	>50 μM
Cyclodidemnimide C Cmpd 70 RAB-005 	inactive	>50 μM	>50 μM	>50 μM
Didemnimide C RAB-006 	inactive	5 μM	1 μM	17 μM
Didemnimide A RAB-004 	inactive	10 μM	0.6 μM	0.6 μM
RAB-011 	inactive	>50 μM	>50 μM	>50 μM

WHAT IS CLAIMED IS:

1. A granulatimide compound having th structure:



or



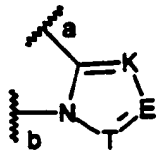
wherein:

F and F' are independently R or Z as defined below, or in combination F and F' is Ar_1 as defined below;

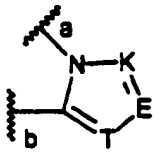
Ar_1 is a monocyclic, bicyclic or tricyclic, fully or partially aromatic system containing five or six membered carbocyclic or, oxygen, nitrogen or sulphur containing heterocyclic rings, optionally substituted with R or Z;

W is selected from the group consisting of formula (i); (ii) or (iii), wherein the structures are as follows:

(i)

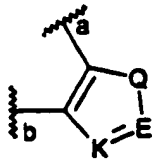


or

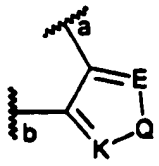


in which K, E and T are independently selected from the group consisting of: N, CR, and CZ, and wherein R and Z are as defined below;

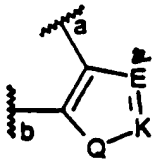
(ii)



or

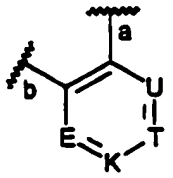


or



in which K and E are independently selected from the group consisting of: N, CR and CZ, and wherein R, Z and Q are as defined below; and

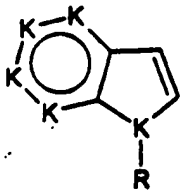
(iii)



5. The granulatimide compound of Claim 3, wherein R_1 is H or CH_3.

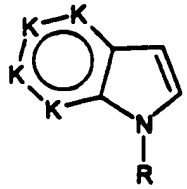
6. The granulatimide compound of Claim 1, wherein X and Y are oxygen.

7. The granulatimide compound of Claim 1, wherein F and F' are in combination, a bicyclic aromatic system comprising a five-membered and a six-membered ring, having the structure:



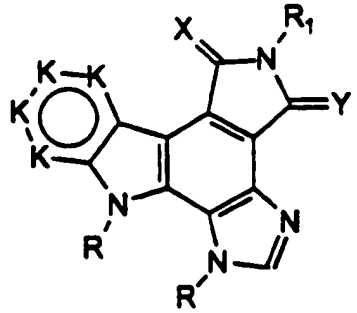
wherein each K is independently is selected from the group consisting of N, CR and CZ; and wherein Z and R are as defined in Claim 1.

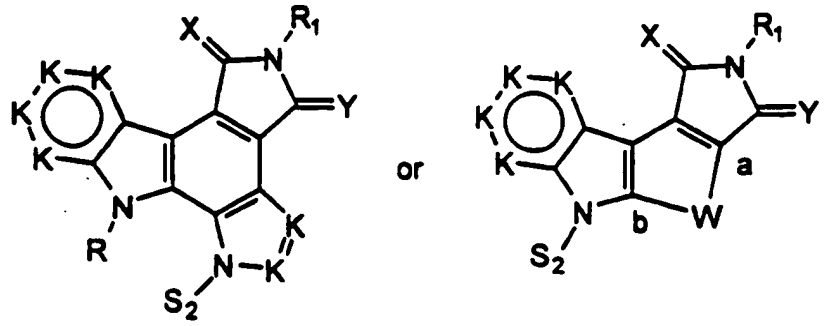
8. The granulatimide compound of Claim 7, wherein F and F' in combination have the bicyclic structure:



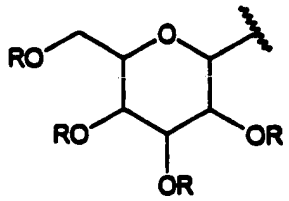
9. The granulatimide compound of Claim 8, wherein W is a five-membered ring of formula (i) or formula (ii).

10. The granulatimide compound of Claim 9, having the structure:





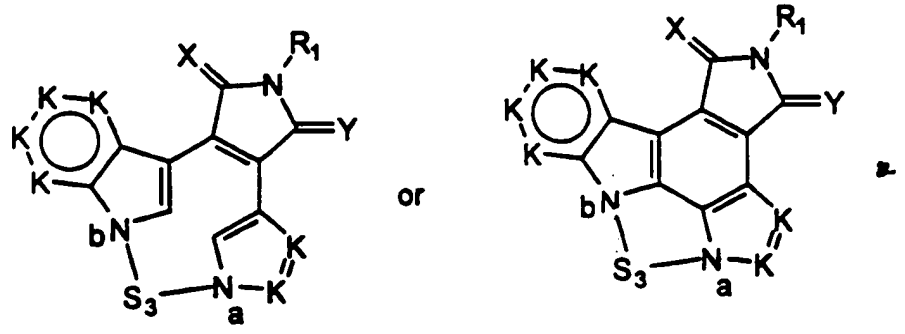
where K, X, Y and R_1 are as defined in Claim 1, R is H or an alkyl of from one to six carbon atoms; and S_2 has the formula (iv)



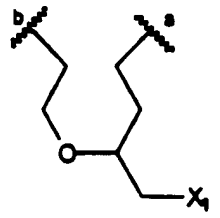
(iv)

or S_2 is a linear alkyl chain of from one to eight carbon atoms containing a terminal NR_2, where R_2 is an alkyl group of from 1 to 6 carbon atoms, branched, linear or cyclic.

13. The granulatimide compound of Claim 10, wherein the compound is further derivatized as follows:



where K, X, Y and R_1 are as defined in Claim 1; S_3 is



20. A method of inhibiting a prot in kinase, th method comprising:

contacting said protein kinase with a dose of a granulatimide compound of Claim 1, in an amount effective to inhibit said protein kinase.

21. A method of treating a condition characterized by a defect in protein kinase mediated signaling, the method comprising:

administering a dose of a granulatimide compound of Claim 1, in an amount effective to inhibit said protein kinase.

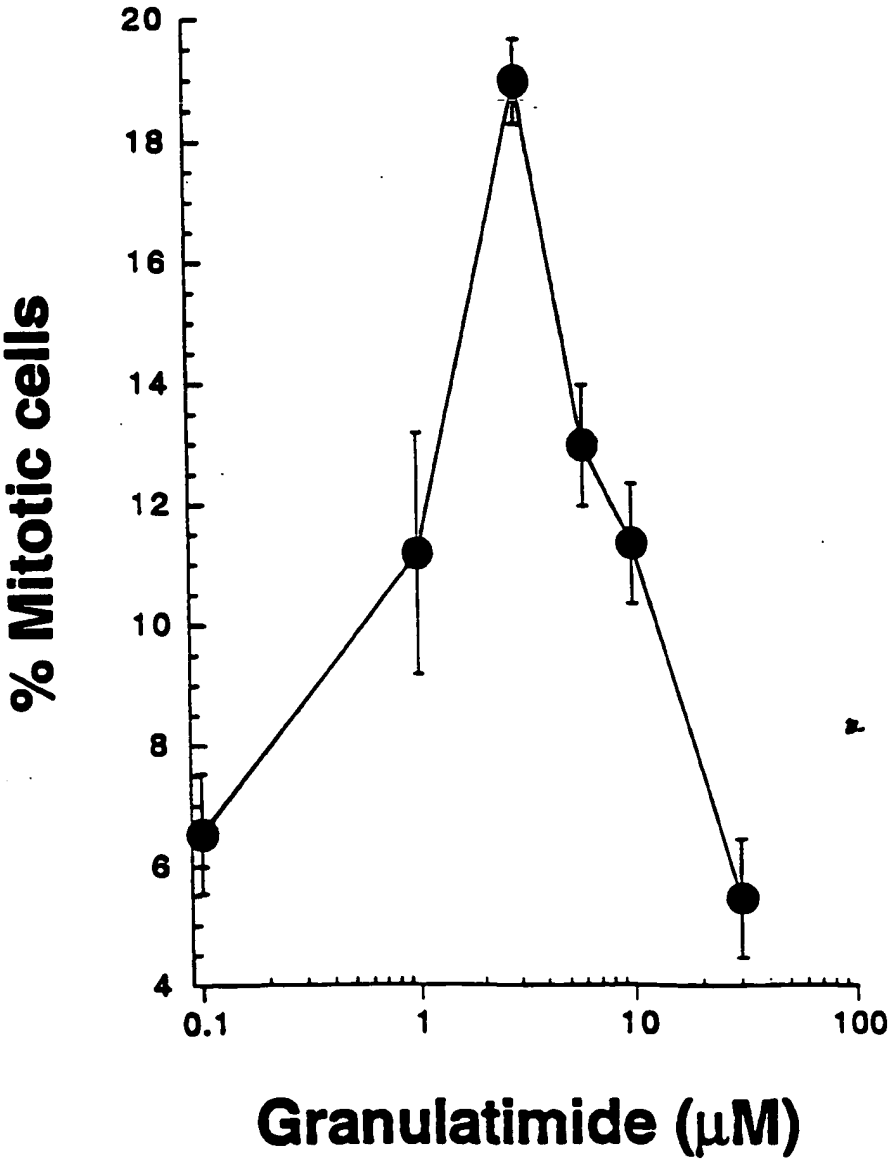

% Mitotic cells
20
18
16
14
12
10
8
6
4
0.1
1
10
100
Granulatimide (μM)

FIGURE 2

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 99/00224

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